

# Topical Application of CO<sub>2</sub> Increases Skin Blood Flow

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The topical application of carbon dioxide water to the rat hindpaw produced a concentration-dependent increase of skin blood flow as measured by a laser Doppler flowmeter. About a 100% increase of skin blood flow occurred in response to CO<sub>2</sub> when the bath temperature was at 23°C or 34°C, but there was no significant effect of CO<sub>2</sub> at 41°C.

Carbon dioxide (CO<sub>2</sub>) applied externally to the skin is well known to produce an erythematous or hyperemic reaction in the human and CO<sub>2</sub> baths have been used for the treatment of peripheral vascular disorders [1–3]. However, not much basic knowledge has been accumulated about the quantitative effect of CO<sub>2</sub> or the mechanism of the effect of CO<sub>2</sub> on the skin vasculature, partly because of lack of suitable animal models. Recently, laser Doppler flowmetry has been established as a useful technique to demonstrate cutaneous blood flow changes [4–9] and has been used to quantitate the vascular effect of CO<sub>2</sub> in the human and rabbits [10,11].

In the present study we have examined the effect of CO<sub>2</sub> exposure on cutaneous blood flow using the rat hindpaw as a model for hairless skin and reported that CO<sub>2</sub> bathing increases the skin blood flow in a concentration related manner at the bathing temperature of 23°C or 34°C and that this effect of CO<sub>2</sub> does not differ among the control, acutely denervated, and chronically denervated animals, indicating no involvement of neuronal mechanism in the vascular effect of CO<sub>2</sub>.

## MATERIALS AND METHODS

Male Sprague-Dawley rats (300–400 g) were anesthetized with pentobarbital and chloral hydrate (40 and 200 mg/kg, i.p., respectively) and were maintained under artificial respiration with room air after tracheal intubation. Cutaneous blood flow was monitored by laser Doppler flowmeters (models Pf2 and Pf3, Perimed, Sweden) from the digital pad of each hindpaw. The flowmeters were calibrated by adjusting the gain to the same reading in millivolts by use of Pf 100 motility standard (Perimed, Piscataway, NJ) and they were used randomly to measure the blood flow either from the control site or CO<sub>2</sub>-bathed sites. To eliminate muscle movement and to obtain stable recordings the animals were paralyzed with gallamine triethiodide (2–10 mg/kg, i.v.), administered through a cannula placed into a jugular vein. A carotid artery also was cannulated to monitor the mean blood pressure by means of a pressure

transducer (Statham P23Dd). The measured skin blood flow and blood pressure were recorded on a Grass 7B polygraph. A bath chamber made of plastic was specially designed with two sections so that both hindpaws of each rat could be lowered into the water individually, allowing one hindpaw to be used as a control for each experiment. Each section of the chamber was filled with 400 ml of distilled water buffered 1:10 with CO<sub>2</sub> buffer solution (Orion Research Inc., Cambridge, MA, 95-02-10). The buffered solution is not only required for use of the CO<sub>2</sub> electrode (see below) but also served to maintain a stable pH (5.25) during the introduction of CO<sub>2</sub> into the solution. The pH did not change when CO<sub>2</sub> was bubbled into the solution. One chamber was bubbled with CO<sub>2</sub> gas at a rate of 50 ml/min through an air stone, whereas the other one remained without bubbling as a control.

Whenever the skin blood flow of the control monitoring site became unstable the experiment was stopped and redone from the beginning after rinsing the chamber and replacing the solutions. The chamber was put on a thermostatically controlled heating unit to keep the water temperature stable at a set temperature [23 ± 0.2°C (low), 34 ± 0.3°C (middle), or 41°C ± 0.6°C (high)] during CO<sub>2</sub> bubbling.

Carbon dioxide concentration was measured using a CO<sub>2</sub> electrode (Ionalyzer, Model 95-02-00, Orion) with a microprocessor ionalyzer (Model-901, Orion). Table I shows the relationship between the time of bubbling with CO<sub>2</sub> (50 ml/min) and the measured CO<sub>2</sub> concentration at the three temperatures used. The effect of temperature on skin blood flow was studied by increasing water temperature continuously from room temperature (22°C–23°C) to 42°C over approximately 70–80 min.

Acute denervation was performed by section of the sciatic and femoral nerves of one hindpaw and the skin blood flow was measured after a 30–45-min period of stabilization. In the chronic denervation experiments, the surgery was performed 5–7 d before the experiment.

Five to 9 rats were used for each experimental group. Results are represented as the means ± SEM. Statistical analysis was performed by Student's *t*-test for unpaired observations.

## RESULTS

The rat hindlimb digital pad showed reasonably stable skin blood flows when the hindlimb was immersed in distilled water at room temperature (22°C–23°C) and flows remained at about the same level up to a temperature of 25°C. At temperatures above 25°C, the skin blood flow increased markedly and the magnitude of increased flow was correlated with the increase of the temperature of the bath up to 42°C (Fig 1). This effect of increasing temperature on skin

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**Table I.** Relationship of Bubbling Time and CO<sub>2</sub> Concentration in the Bathing Water at Various Temperatures

Time (min)	Water Temperature		
	23°C	34°C	41°C
3	366 ± 32	367 ± 28	365 ± 39
6	740 ± 56	704 ± 79	705 ± 64
9	1165 ± 84	932 ± 103	894 ± 61
12	1483 ± 77	1149 ± 119	1102 ± 64
15	1808 ± 75	1326 ± 89	1276 ± 60
20	2133 ± 79	1498 ± 86	1450 ± 48

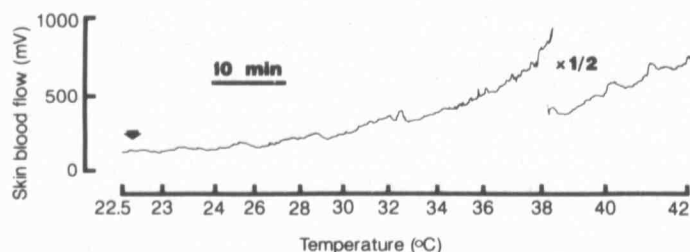
Values represent mean ± SEM of CO<sub>2</sub> concentration in ppm. n = 5 for each value.

blood flow was not significantly different among the control, acutely denervated, or chronically denervated groups. The maximal effect of temperature on skin blood flow appeared to increase flow by approximately 600% or more (Fig 2). Basal skin blood flow measurements (laser Doppler flowmeter readings in mV) before heating at 24°C were 164.4 ± 23.7 in the control, 129.0 ± 10.7 in the acutely denervated, and 198.0 ± 45.1 in the chronically denervated animals and after heating at 42°C flows were 1107.8 ± 155.4, 1134.5 ± 169.2, and 1294.0 ± 176.9, respectively. When comparing denervated groups with the controls, no significant difference was observed. There was, however, a significant difference between the acutely and chronically denervated animals at 24°C ( $p < 0.05$ ).

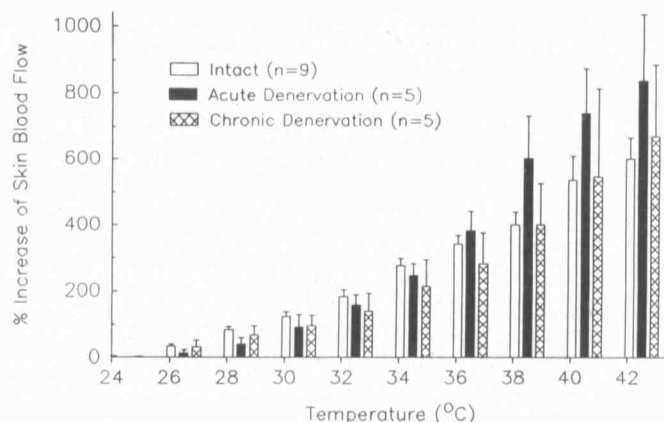
Carbon dioxide gas bubbled into the bath water produced an increase in the cutaneous blood flow. A minimal effect was observed after about 3 min of CO<sub>2</sub> bubbling and the maximal response occurred in about 20 min while the skin blood flow of the control remained unchanged (Fig 3). Figure 4 illustrates typical skin blood flow changes in the acutely denervated paw at three different temperatures. As can be seen, CO<sub>2</sub> elicited about a 100% increase of the skin blood flow at the low and middle temperatures, but did not show a significant effect at the high temperature. As shown in Figures 5 and 6, this effect of CO<sub>2</sub> to increase skin blood flow at the low or middle temperature was not significantly different among control, acutely denervated, and chronically denervated animals. In these groups, CO<sub>2</sub> did not appreciably increase skin blood flow at the high temperature. Composite results from the three experimental groups of actual laser Doppler flowmeter readings before and 20 min after CO<sub>2</sub> bubbling are shown in Table II. Bubbling with room air up to a rate of 200 ml/min did not alter skin blood flow.

## DISCUSSION

Carbon dioxide bathing has an old history, especially in Europe, and is thought to be effective in the treatment of peripheral vascular diseases, hypertension, and heart disease [1-3,12-14]. The effect of CO<sub>2</sub> bathing to increase cutaneous blood flow has been investigated mainly in humans and there have not been many experimental studies to determine the mechanism(s) of the CO<sub>2</sub> effect. It has been



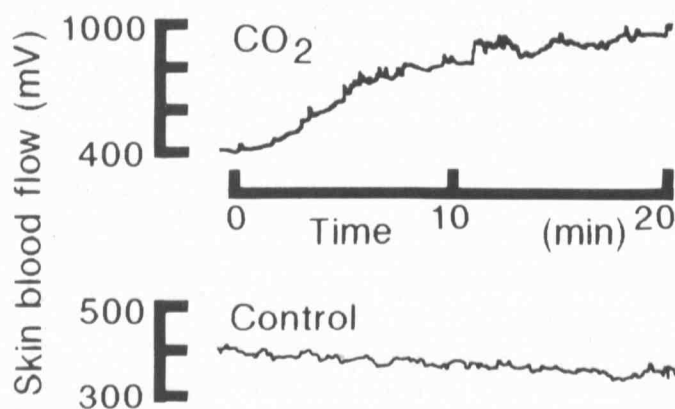
**Figure 1.** Effect of increasing bathing water temperature on the skin blood flow of a rat hindlimb digital pad as measured by a laser Doppler flowmeter (in mV, ordinate). Innervation to the vasculature was intact in this preparation. Note the recording sensitivity change at 38°C.



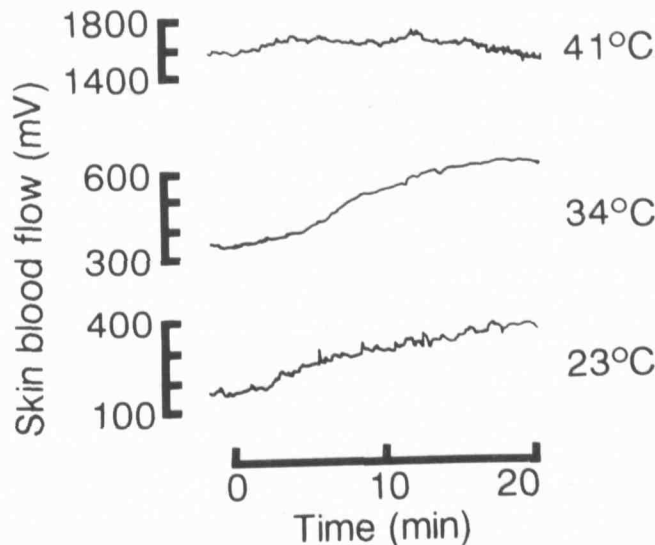
**Figure 2.** Composite representation of the effect of water temperature on the cutaneous blood flow of the rat hindlimb digital pad. The ordinate represents percentage of increase of laser Doppler flowmeter readings from the control level before heating. Values represent mean ± SEM. There were no significant differences between the three experimental groups.

reported that CO<sub>2</sub> bathing might exhibit a diuretic effect by decreasing sympathetic tone in humans [15]. In contrast, Bühring et al [16] recently observed that CO<sub>2</sub> has an antidiuretic effect in temperature-matched bathing experiments in humans. These investigators proposed as a possible mechanism that blood pooling in the skin, produced by the vasodilating effect of CO<sub>2</sub>, reduces renal circulation to cause less urine formation [16]. The present results did not show a significant difference of the effect of CO<sub>2</sub> to increase cutaneous blood flow among the intact, acutely, or chronically denervated groups of animals. This would appear to indicate that there is no involvement of a neural mechanism, at least in the local effect of CO<sub>2</sub>, on skin blood flow. This finding is in agreement with the theory proposed by Jordan [17] that CO<sub>2</sub> is acting as a first messenger directly on the vascular smooth muscle cells or that the effect of CO<sub>2</sub> on the skeletal muscle is to reduce the tension through decreasing intracellular pH [18].

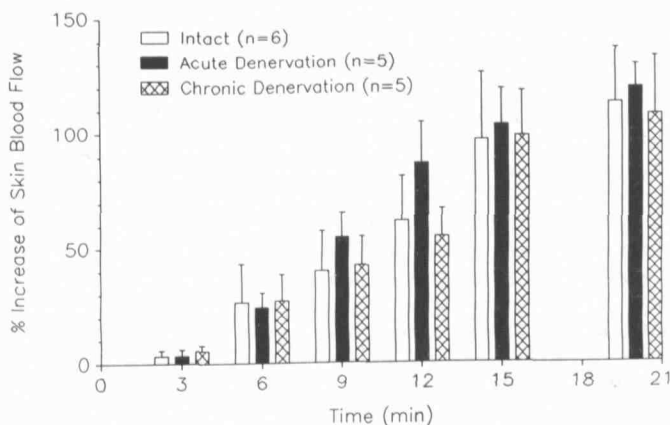
It is well known that increase of PCO<sub>2</sub> or decrease of PO<sub>2</sub> in blood can also dilate blood vessels. The PCO<sub>2</sub> in mixed venous blood and the amount of CO<sub>2</sub> exhaled can be increased when CO<sub>2</sub> water is applied to the whole body of humans [19]. In the present study, however, we did not see any increase of the blood flow on the control paw. This observation suggests that the change of systemic



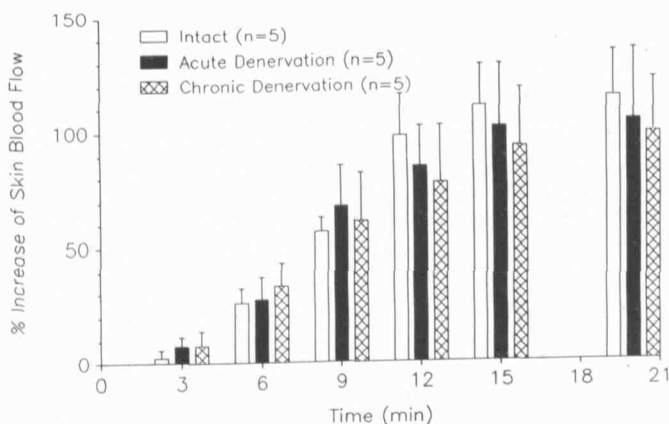
**Figure 3.** Effect of CO<sub>2</sub> on the cutaneous blood flow of a rat hindlimb digital pad with intact innervation. The CO<sub>2</sub> was bubbled into the bathing water using an air stone (50 ml/min). Time from the commencement of bubbling is shown in minutes (upper panel). Lower panel represents the control skin blood flow from the other hindpaw immersed into distilled water without CO<sub>2</sub> bubbling. Both paws were innervated. Water temperature was held constant at 34°C.



**Figure 4.** Effect of CO<sub>2</sub> on the cutaneous blood flow of an acutely denervated hindlimb digital pad of a rat at different bathing water temperatures (23°C, 34°C, and 41°C). The abscissa represents the time from the beginning of CO<sub>2</sub> bubbling into the bathing water (50 ml/min).



**Figure 5.** Effect of CO<sub>2</sub> on the cutaneous blood flow measured at 23°C. The abscissa represents time of CO<sub>2</sub> bubbling. The final set of bars represents measurements taken at 20 min. No significant differences were seen between three experimental groups at each time of measurement. Values represent mean  $\pm$  SEM.



**Figure 6.** Effect of CO<sub>2</sub> on the cutaneous blood flow measured at 34°C. The final set of bars represents measurements taken at 20 min. No significant differences were seen between three experimental groups at each time of measurement. Values represent mean  $\pm$  SEM.

**Table II.** Effect of CO<sub>2</sub> on Skin Blood Flow of the Rat Hindlimb Digital Pad (Laser Doppler Flowmeter Readings in mV)

Temperature		Intact Nerves	Acutely Denervated	Chronically Denervated
23°C	<sup>a</sup> B	147 $\pm$ 41	205 $\pm$ 52	140 $\pm$ 17
	<sup>b</sup> A	304 $\pm$ 53	454 $\pm$ 128	280 $\pm$ 14
34°C	B	344 $\pm$ 50	472 $\pm$ 142	606 $\pm$ 217
	A	758 $\pm$ 167	864 $\pm$ 199	1050 $\pm$ 292
41°C	B	1784 $\pm$ 365	1362 $\pm$ 143	1498 $\pm$ 293
	A	1874 $\pm$ 311	1412 $\pm$ 104	1410 $\pm$ 241

<sup>a</sup> Before and <sup>b</sup> after 20 min of CO<sub>2</sub> bubbling (50 ml/min). Values represent readings of laser Doppler flowmeter in mV with mean  $\pm$  SEM. Five or 6 animals per group.

Pco<sub>2</sub> or Po<sub>2</sub> is not a causative factor in the skin blood flow response to CO<sub>2</sub> bathing in our experiments.

Another point of interest in the present study is the relationship of the effect of temperature to that of CO<sub>2</sub> on cutaneous blood flow. Our results showed that CO<sub>2</sub> increases skin blood flow about 100% above basal flow at both 23°C and 34°C, but laser Doppler flowmeter readings reveal that the absolute amplitude of the increase in cutaneous blood flow at 34°C is more than two times of that at 23°C (Table II). The lack of CO<sub>2</sub> effect on skin blood flow at high temperature (41°C) might be explained by the more apparent effect of high temperature itself. If the maximum increase of blood flow was attained by high temperature alone, CO<sub>2</sub> would be unable to increase flow further. We have no information concerning the mechanism of the interaction between temperature change and CO<sub>2</sub> effects, but there might be a certain temperature where CO<sub>2</sub> can produce the greatest effect on the cutaneous blood flow.

From the clinical aspect the present observations provide experimental support for the benefit of CO<sub>2</sub> bathing in the treatment of skin circulatory disorders. Although the effect of high temperature on skin blood flow is more obvious than that of CO<sub>2</sub> itself, possibly the effectiveness of CO<sub>2</sub> at the temperature of 34°C, which is close to the neutral temperature, may help the healing of skin wounds and chronic skin ulcers. This might be particularly true in cases like livedo reticularis with summer ulceration, where increased temperature is suspected to cause skin ulceration by increasing tissue O<sub>2</sub> demand [20]. The increase of blood flow in the denervated skin by CO<sub>2</sub> bathing also would be advantageous for wound healing in patients with nerve disturbances of the skin as neural mechanisms are not needed for the effect of CO<sub>2</sub> to be manifest.

Carbon dioxide bathing is thought to manifest its effect on degenerative or tropic diseases partly through increasing tissue O<sub>2</sub> tension or tissue metabolism [10]. In this sense, CO<sub>2</sub> bathing might have an effect similar to other methods (e.g., hyperbaric, ultrasound, or microwave). With its action to increase skin blood flow and to improve tissue metabolism, CO<sub>2</sub> bathing might be a useful clinical method to treat skin wounds and ulcers or skin circulatory disorders with trophic changes.

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